

Effective free energy for pinned membranes

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We consider membranes adhered through specific receptor-ligand bonds. Thermal undulations of the membrane induce effective interactions between adhesion sites. We derive an upper bound to the free energy that is independent of interaction details. To lowest order in a systematic expansion we obtain two-body interactions which allow to map the free energy onto a lattice gas with constant density. The induced interactions alone are not strong enough to lead to a condensation of individual adhesion sites. A measure of the thermal roughness is shown to depend on the inverse square root of the density of adhesion sites, which is in good agreement with previous computer simulations.

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Living cells interact with their environment through the cell membrane, a bilayer of phospholipids consisting of hydrophobic tails and hydrophilic heads. Embedded integral proteins, which make up a large portion of the membrane, are involved in a range of processes such as ion passage and cell signaling. Through the formation of receptor-ligand bonds certain proteins such as cadherins, selectins, and integrins are involved in cell adhesion, where one cell binds to another cell, to an extracellular matrix, or to a surface [1]. Extensive studies have been conducted on cell mimetic model systems using a variety of techniques [2–5].

The proteins embedded into the membrane can move rather freely together with the lipids. The lateral dynamics and diffusion properties of embedded integral proteins have been studied both theoretically [6, 7] and experimentally [8]. Protein dynamics is influenced by the cytoskeleton, geometric constraints, and the thermal undulations of the membrane itself. Adhesion sites (i.e., single receptor-ligand bonds) in cells are not randomly distributed but form focal adhesions which are several square microns in area [9]. Since the bending of the membrane requires energy one would imagine that such a clustering of adhesion sites is preferential. However, computer simulations of confined membranes [10–13] indicate that fluctuation-induced interactions between adhesion sites are short-ranged and insufficiently strong to lead to thermodynamically stable clusters, quite in contrast to earlier predictions [14, 15]. The aim of this Rapid Communication is to derive an effective and accurate expression for the interaction free energy of pinned membranes through a variational approach. Membrane-induced interactions between adhesion sites are indeed found to be rather weak, confirming that active processes and inter-protein interactions are primarily responsible for the formation of adhesion domains.

We consider a tension-less, almost flat membrane moving close to a substrate in a fluid at temperature T . The membrane height profile $h(\mathbf{r})$ is given in the Monge representation with $\mathbf{r} = (x, y)$. The projected area is L^2 and for simplicity we employ periodic boundary conditions. In this geometry the membrane can be thought of as a two dimensional sheet, where the thermal fluctuations

are governed by the Helfrich energy [16]. We focus on the bending energy

$$\mathcal{H}_0[h(\mathbf{r})] = \int d^2\mathbf{r} \frac{\kappa}{2} [\nabla^2 h(\mathbf{r})]^2 \quad (1)$$

as the dominant contribution with bending rigidity κ . In the presence of N adhesion centers at locations $\{\mathbf{r}_i\}$ pinning the membrane to the substrate the partition sum reads

$$\mathcal{Z}_N(\{\mathbf{r}_i\}) \equiv \int [dh(\mathbf{r})] e^{-\beta(\mathcal{H}_0 + \mathcal{H}_i)} \prod_{i=1}^N \ell \delta(h(\mathbf{r}_i)) \quad (2)$$

with $\beta \equiv (k_B T)^{-1}$. The path integral sums over all accessible membrane configurations $h(\mathbf{r}) \geq 0$. The (small) length ℓ is related to the flexibility of bonds and quantifies the fluctuations of the membrane at the adhesion sites (one should think of the δ -functions as limits of Gaussians). Van der Waals forces, the repulsion due to the formation of an electrostatic double-layer, and steric

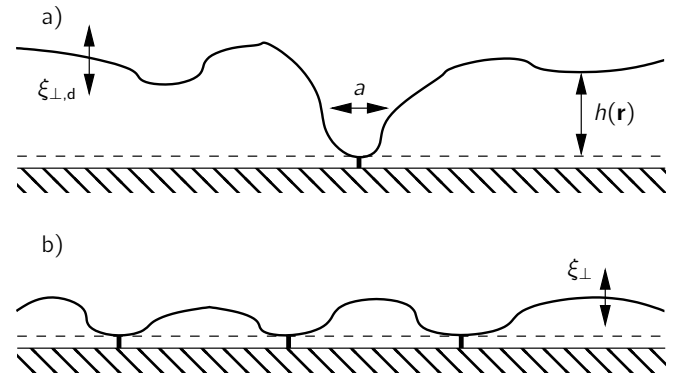


FIG. 1: Sketch of a membrane with instantaneous height profile $h(\mathbf{r})$ moving above a substrate. The membrane is pinned down through adhesion centers with effective size a . a) In the presence of only a few centers the thermal roughness away from the adhesion centers is $\xi_{\perp,d}$. b) For a large number of adhesion centers the membrane is pulled closer to the substrate. This reduces the thermal roughness $\xi_{\perp} < \xi_{\perp,d}$ due to interactions of the membrane with the substrate.

forces give rise to an interaction energy $\mathcal{H}_i[h(\mathbf{r})]$ between substrate and membrane, see Fig. 1. While explicit expressions can be found for these forces [17], here we pursue a more general strategy that does not depend on specific details.

We expand the height profile $h(\mathbf{r})$ into Fourier modes,

$$h(\mathbf{r}) = \sum_{\mathbf{q}} h_{\mathbf{q}} e^{-i\mathbf{q}\cdot\mathbf{r}}, \quad h_{\mathbf{q}} = \frac{1}{L^2} \int d^2\mathbf{r} h(\mathbf{r}) e^{i\mathbf{q}\cdot\mathbf{r}},$$

where $h_{\mathbf{q}}^* = h_{-\mathbf{q}}$. In particular, h_0 is the height averaged over the area of the membrane. The Helfrich Hamiltonian (1) becomes

$$\mathcal{H}_0 = \frac{\kappa L^2}{2} \sum_{\mathbf{q}} q^4 |h_{\mathbf{q}}|^2 = \frac{1}{2} \sum_{\mathbf{q} \in \mathcal{Q}} 2\kappa L^2 q^4 [h_{\mathbf{q}}'^2 + h_{\mathbf{q}}''^2],$$

where we split $h_{\mathbf{q}} = h_{\mathbf{q}}' + ih_{\mathbf{q}}''$ into real and imaginary part and $q \equiv |\mathbf{q}|$. We sum only over independent wave vectors, i.e., for a given \mathbf{q} the set \mathcal{Q} does not contain $-\mathbf{q}$. Also, $q = 0$ is excluded from the sum.

Due to the pinning the membrane is pulled towards the substrate. The interactions of the membrane with the substrate lead to a 'confinement' of fluctuations. For membranes placed between walls the interaction energy is often expanded into a quadratic function of the height. However, in principle we can do better through introducing a quadratic reference energy \mathcal{H}_r that acts on every height mode independently. Employing the Jensen inequality we know that the free energy is bounded from above as [18]

$$\mathcal{F}_N \leq \tilde{\mathcal{F}}_N + \langle \Delta \mathcal{H} \rangle \equiv F_N \quad (3)$$

with $\mathcal{F}_N \equiv -\beta^{-1} \ln \mathcal{Z}_N$. Here, $\Delta \mathcal{H} \equiv \mathcal{H}_i - \mathcal{H}_r$ and $\langle \dots \rangle$ denotes the average with respect to the reference system with partition sum $\tilde{\mathcal{Z}}_N$ and free energy $\tilde{F}_N \equiv -\beta^{-1} \ln \tilde{\mathcal{Z}}_N$. By minimizing the function F_N with respect to a set of free parameters we obtain an approximation of the true free energy $\mathcal{F}_N(\{\mathbf{r}_i\})$. In this work we choose an intermediate description

$$\mathcal{H}_r \equiv \frac{1}{2} \gamma_0 (h_0 - \bar{h})^2 + 2L^2 \gamma \sum_{\mathbf{q} \in \mathcal{Q}} [h_{\mathbf{q}}'^2 + h_{\mathbf{q}}''^2] \quad (4)$$

with free parameters $\{\gamma_0, \bar{h}, \gamma\}$. The total energy then reads

$$\beta(\mathcal{H}_0 + \mathcal{H}_r) = \frac{1}{2} \hat{\gamma}_0 (h_0 - \bar{h})^2 + \frac{1}{2} \sum_{\mathbf{q} \in \mathcal{Q}} \hat{\gamma}_{\mathbf{q}} [h_{\mathbf{q}}'^2 + h_{\mathbf{q}}''^2] \quad (5)$$

with $\hat{\gamma}_0 \equiv \beta \gamma_0$ and $\hat{\gamma}_{\mathbf{q}} \equiv 2L^2 \beta (\gamma + \kappa q^4)$.

Before we calculate the reference partition sum $\tilde{\mathcal{Z}}_N$ let us assume we had performed the minimization of F_N in the presence of the adhesion centers with constraints $h(\mathbf{r}_i) = 0$. We hypothetically remove the constraints but keep the values for γ_0 , \bar{h} , and γ we had obtained as a

result of the minimization. We define the thermal roughness ξ_{\perp} through

$$\xi_{\perp}^2 \equiv \langle [h(\mathbf{r}) - h_0]^2 \rangle_0 = \sum_{\mathbf{q} \neq 0} \langle |h_{\mathbf{q}}|^2 \rangle_0 = 4 \sum_{\mathbf{q} \in \mathcal{Q}} \frac{1}{\hat{\gamma}_{\mathbf{q}}}, \quad (6)$$

which is independent of γ_0 and \bar{h} . The brackets $\langle \dots \rangle_0$ denote the average involving the energy Eq. (5) in the absence of constraints. For the sake of simplicity and for sufficiently large L we evaluate the sum over wave vectors \mathbf{q} through a two-dimensional integral over the half-plane with the well-known result

$$\xi_{\perp} \approx \frac{\pi L^2}{(2\pi)^2} \int_{2\pi/L}^{\infty} dq q \frac{4}{\hat{\gamma}_q} \approx \xi_{\parallel} / \sqrt{8\beta\kappa} \leq \xi_{\perp,d} \quad (7)$$

in the limit $L \gg \xi_{\parallel}$. Here, $\xi_{\parallel} \equiv (\kappa/\gamma)^{1/4}$ is the lateral decay length over which membrane fluctuations are correlated. The thermal roughness is bounded by its value $\xi_{\perp,d} \approx L/\sqrt{16\pi^3\beta\kappa}$ for a free, detached membrane with $\gamma = 0$. Note that the thermal roughness ξ_{\perp} obtained by averaging over the projected area including the adhesion sites is greater than ξ_{\perp} .

We integrate over the membrane height fluctuations in the presence of N adhesion centers at positions $\{\mathbf{r}_i\}$ [14, 19]. The reference partition sum including the constraints reads

$$\begin{aligned} \tilde{\mathcal{Z}}_N &= (\ell/2\pi)^N \int [dh(\mathbf{r})] e^{-\beta(\mathcal{H}_0 + \mathcal{H}_r)} \int d\lambda_1 \dots d\lambda_N \\ &\times \exp \left\{ i h_0 \sum_{i=1}^N \lambda_i + i \sum_{\mathbf{q} \in \mathcal{Q}} [c'_{\mathbf{q}} h'_{\mathbf{q}} + c''_{\mathbf{q}} h''_{\mathbf{q}}] \right\} \quad (8) \end{aligned}$$

with coefficients

$$c'_{\mathbf{q}} \equiv 2 \sum_{i=1}^N \lambda_i \cos \mathbf{q} \cdot \mathbf{r}_i, \quad c''_{\mathbf{q}} \equiv 2 \sum_{i=1}^N \lambda_i \sin \mathbf{q} \cdot \mathbf{r}_i.$$

We have replaced the δ -constraints [Eq. (2)] by integrals over a set of auxiliary variables $\{\lambda_i\}$. We assume that the excluded volume constraint is represented by the interaction energy \mathcal{H}_i , and therefore by \mathcal{H}_r . Performing the integrations we obtain

$$\tilde{\mathcal{Z}}_N \sim \tilde{\mathcal{Z}}_0 [\det m / \ell^{2N}]^{-1/2}, \quad \tilde{\mathcal{Z}}_0 = \prod_{\mathbf{q} \in \mathcal{Q}} \frac{1}{\hat{\gamma}_{\mathbf{q}}}, \quad (9)$$

where we have dropped the sub-extensive contribution due to the integration over h_0 . The symmetric $N \times N$ matrix m has components $m_{ij} \equiv m(|\mathbf{r}_i - \mathbf{r}_j|)$, where

$$m(r) \equiv 4 \sum_{\mathbf{q} \in \mathcal{Q}} \frac{\cos \mathbf{q} \cdot \mathbf{r}}{\hat{\gamma}_{\mathbf{q}}} \approx \xi_{\perp}^2 u(r/\xi_{\parallel}) \quad (10)$$

describes the membrane-mediated interactions. The spatial correlations between height fluctuations and therefore the effective lateral interactions between adhesion sites is short ranged with potential $u(x) \equiv$

$-(4/\pi) \text{kei}_0(x)$, where $\text{kei}_0(x)$ is a Kelvin function [20]. The effective interactions decay on the single length scale ξ_{\parallel} that also determines the thermal roughness ξ_{\perp} [Eq. (7)].

For the calculation of F_N [Eq. (3)] we first determine the contribution

$$-\ln \tilde{Z}_0 = \sum_{\mathbf{q} \in \mathcal{Q}} \ln \hat{\gamma}_{\mathbf{q}} = L^2/(8\xi_{\parallel}^2), \quad (11)$$

which for small ξ_{\parallel} captures the loss of entropy due to a large confinement of fluctuations. To establish F_N as a rigorous upper bound to the free energy \mathcal{F}_N we impose

$$\beta \langle \Delta \mathcal{H} \rangle \leq -\beta \langle \mathcal{H}_r \rangle_0 = -L^2/(4\xi_{\parallel}^2). \quad (12)$$

This bound restricts the accessible values of γ_0 and \bar{h} . We assume that the energy $\langle \mathcal{H}_r \rangle_0$ required to constrain the fluctuations is the dominant contribution to $\langle \Delta \mathcal{H} \rangle$. The equal sign holds if the interaction energy $\langle \mathcal{H}_i \rangle$ is exactly balanced by $\langle \mathcal{H}_r \rangle - \langle \mathcal{H}_r \rangle_0$ describing the energy required to bend (and, in principle, stretch) the membrane around the adhesion centers. Putting together Eqs. (9)-(12) we see that the resulting upper bound $F_N(\xi_{\parallel}; \{\mathbf{r}_i\})$ is a function of ξ_{\parallel} alone and independent of interaction details between membrane and substrate, which is our first main result. In the following we use the minimum $F_N(\xi_{\parallel}^*)$ as an approximation to the free energy. We split $F_N = Nf + F_N^{\text{ex}}$ into an ideal part f and an excess free energy F_N^{ex} . Introducing an effective size a of adhesion centers with area fraction $\phi \equiv Na^2/L^2$ the ideal part reads

$$\beta f(\xi_{\parallel}) \equiv \frac{(a/\xi_{\parallel})^2}{16\phi} + \ln(\xi_{\parallel}/a) + \ln(a/\xi_0) \quad (13)$$

with $\xi_0 \equiv \sqrt{8\beta\kappa}\ell$. The excess free energy becomes

$$\beta F_N^{\text{ex}}(\xi_{\parallel}; \{\mathbf{r}_i\}) \equiv \frac{1}{2} \ln \det \chi \quad (14)$$

due to the membrane-induced interactions between adhesion sites. These interactions are encoded in the matrix χ with elements

$$\chi_{ij}(\xi_{\parallel}; \{\mathbf{r}_i\}) = \begin{cases} 1 & i = j, \\ u(|\mathbf{r}_i - \mathbf{r}_j|/\xi_{\parallel}) & i \neq j. \end{cases} \quad (15)$$

Using $\ell < \xi_{\perp} \leq \xi_{\perp, \text{d}}$ we find the meaningful range

$$\xi_0 < \xi_{\parallel} \leq \frac{1}{\sqrt{2\pi^3}} L \simeq 0.13L \quad (16)$$

for the decay length ξ_{\parallel} of the effective interactions. For large ξ_{\parallel} we expect a crossover to free fluctuations while below ξ_0 the membrane is effectively so stiff that a translation of adhesion centers does not require to bend the membrane. Using Eq. (12) the temperature and bending rigidity $\beta\kappa$ only set this crossover length and do not influence the value ξ_{\parallel}^* that minimizes F_N .

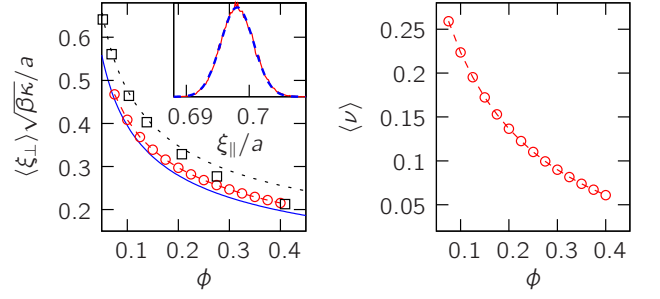


FIG. 2: (color online) Left panel: the mean thermal roughness $\langle \xi_{\perp} \rangle$ vs. area fraction ϕ for the lattice gas (circles) and the non-interacting adhesion centers [Eq. (17), solid line]. For comparison the *global* thermal roughness reported in Ref. [10] is shown (squares) with fit $\bar{\xi}_{\perp} \simeq 0.14\phi^{-1/2} + 0.035$ (dashed line). Inset: probability distribution (solid line) of ξ_{\parallel} for the lattice gas ($\phi = 0.3$), which is well described by a Gaussian (dashed line). Right panel: the mean effective interaction strength $\langle \nu \rangle$. The standard deviation of ν is smaller than symbols.

We first consider non-interacting adhesion sites, i.e., the off-diagonal elements of χ vanish and $F_N^{\text{ex}} = 0$. Minimizing $f(\xi_{\parallel})$ leads to the result

$$\xi_{\parallel}^* = \frac{a}{\sqrt{8\phi}}, \quad \xi_{\perp}^* = \frac{1}{8\sqrt{\phi}} \frac{a}{\sqrt{\beta\kappa}}. \quad (17)$$

Such a scaling of the thermal roughness with the inverse square root of the area fraction has been found in computer simulations [10]. Moreover, the prefactor $\simeq 0.14$ for $\bar{\xi}_{\perp}$ determined numerically in Ref. [10] compares favorably with our $1/8$, see also Fig. 2. Of course, the divergence for $\phi \rightarrow 0$ is only apparent since we expect a crossover to free fluctuations with $\xi_{\perp} \simeq \xi_{\perp, \text{d}}$ if the membrane is pinned by only a few adhesion centers.

In general $\xi_{\parallel}^* = \xi_{\parallel}^*(\{\mathbf{r}_i\})$ will depend on the geometry of adhesion sites and, therefore, is a fluctuating quantity for mobile sites, i.e., ligands are able to move in the substrate (e.g., through using a supported lipid bilayer as substrate). The effective interactions between adhesion sites are attractive ($u > 0$) and one expects a clustering of sites. This will lead to a larger thermal roughness ξ_{\perp} compared to the non-interacting case. To study the question whether these attractions can actually lead to condensation we turn to the simplified case of adhesion centers moving on a square lattice with N_S sites and lattice constant a . The area fraction is $\phi = N/N_S$. We define a symmetric $N \times N$ link matrix b with entries $b_{ij} = 1$ if the two centers i and j are neighbors and $b_{ij} = 0$ otherwise. Then $\chi_{ij} = \delta_{ij} + u_1 b_{ij}$ with $u_1 \equiv u(a/\xi_{\parallel}) < 1$. The number of links is $N_L = (1/2) \sum_{ij} b_{ij}$. Expanding the excess free energy Eq. (14) to second order in u_1 leads to $\beta F_N^{\text{ex}} \approx -\nu N_L$ with interaction strength $\nu \equiv u_1^2/2$. This approximation implies only nearest neighbor interactions and holds for $\xi_{\parallel} < a$. Hence, the statistics of the adhesion centers can be inferred from a two-dimensional lattice gas with constant density (or, equivalently, the Ising model

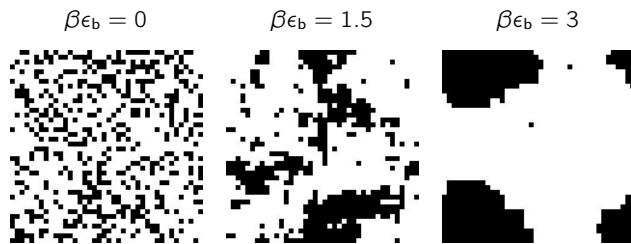


FIG. 3: Three snapshots of the lattice gas (adhesion sites are black squares) for area fraction $\phi = 0.3$ and different values of the binding energy ϵ_b . The snapshots have been taken after 10^5 sweeps starting from random configurations.

with constant magnetization) and Kawasaki diffusion dynamics. This mapping constitutes the second main result of this Rapid Communication. However, in contrast to the standard lattice gas the interaction strength ν is not constant but depends, as does ξ_{\parallel} , on the number of links N_L .

We have performed Monte Carlo simulations of the lattice gas with $N_S = 40^2$. In Fig. 2 we show the averaged thermal roughness $\langle \xi_{\perp} \rangle$ as a function of the area fraction ϕ for both the lattice gas and the ideal non-interacting case [Eq. (17)]. For the lattice gas the decay length ξ_{\parallel} is larger due to the clustering of adhesion centers, which implies that membrane regions can move further away from the substrate exhibiting a larger thermal roughness as compared to the non-interacting case. The decay length fluctuates with a narrow distribution that is Gaussian to a very good degree. For comparison the thermal roughness ξ_{\perp} as obtained numerically in Ref. [10] is plotted. Also shown in Fig. 2 is the interaction strength ν vs. the area fraction. Since for a larger number of adhesion centers the membrane is pulled closer to the substrate the thermal roughness, and therefore the interaction strength, decrease. The effective interaction strength is purely entropic as one would of course expect. Although the interaction strength is not constant its distribution is rather narrow and hence we compare

its mean value $\langle \nu \rangle$ to the critical value $\nu_c \approx 1/2.27 \approx 0.44$ of the standard Ising model. We see that $\langle \nu \rangle < \nu_c$ except for very small area fractions for which we expect the crossover to unconfined fluctuations. The fact that the simulations show no condensation (see Fig. 3 left panel) is therefore due to the fact that the interaction strength is too small. This situation changes if we allow for short-ranged interactions with a binding energy ϵ_b between ligands or receptors or both. Such a binding energy might again be entropic in nature, e.g., due to hydrophobic mismatch [21] or different types of receptors with different lengths [22]. For large enough $\nu = u_1^2/2 + \beta\epsilon_b$ we then observe coarsening and condensation of bonds into large clusters, see Fig. 3.

In summary, we have developed an analytic theory for the statistics of mobile adhesion centers (single receptor-ligand bonds) in adhered membranes. To this end we have established an upper bound to the free energy that is independent of details of the interactions between membrane and substrate. For sites moving on a discrete lattice the resulting minimized free energy can be mapped onto a lattice gas with constant density but fluctuating interaction strength. The mean interaction strength is shown to be well below the critical value of the Ising model. While we have focused on membrane-substrate interactions conceptually the same situation arises if the membrane is moving close to a soft surface such as another cell membrane. Then $h(\mathbf{r})$ denotes the inter-membrane distance and κ is to be replaced by the effective bending rigidity $\kappa = \kappa_1\kappa_2/(\kappa_1 + \kappa_2)$, where κ_i are the bending rigidities of the two membranes.

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